Conclusions: These data suggest that lubricin mRNA and protein expression by synoviocytes is diminished by pro-inflammatory cytokines such as IL-1 beta, and upregulated by anabolic cytokines such as TGF-beta. Increased chondrocyte expression of lubricin was observed after treatment with OSM, typically regarded as a proinflammatory cytokine. For IL-1 beta and TGF-beta, trends in lubricin synthesis by synoviocytes follow those observed for chondrocytes in this and other studies. Therefore, it may be speculated that amounts of lubricin within the synovial joint, both soluble (synovial fluid) and adsorbed (joint surfaces), are regulated by the biosynthetic responses of synoviocytes as well as chondrocytes. Such regulation could conceivably extend to other lubricin-secreting cell types within the synovial joint such as tenocytes and meniscal fibroblasts. It will be interesting to see whether the cytokine-mediated changes in lubricin biosynthesis observed in this study result in relevant functional alterations at the tissue level.

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EXPRESSION PROFILING OF METALLOPROTEINASES AND THEIR INHIBITORS IN SYNOVIUM AND CARTILAGE

R.K. Davidson[1], J.G. Waters[1], L. Kevorkian[1], C. Darrah[2], A. Cooper[2], S.T. Donell[2], I.M. Clark[1]
[1]University of East Anglia, Norwich, United Kingdom, [2]Norfolk & Norwich University Hospital, Norwich, United Kingdom

Purpose: Cartilage destruction in osteoarthritis (OA) is thought to be mediated by two main enzyme families; the matrix metalloproteinases (MMPs) are responsible for cartilage collagen breakdown, whilst enzymes from the 'a disintegrin and metalloproteinase domain with thrombospondin motifs' (ADAMTS) family mediate cartilage aggrecan loss. Tissue inhibitors of metalloproteinases (TIMPs) regulate the activity of these enzymes. Whilst cartilage destruction in OA may be driven by the chondrocyte, low-grade synovitis is reported in patients with all grades of this disease. Our earlier work profiling these gene families in cartilage identified a number of genes regulated in OA which are hence implicated in the disease process. Since the synovium may contribute to cartilage matrix destruction in OA, we have extended this screen in the current study. We have profiled *MMPs*, *ADAMTSs* and *TIMPs* in both cartilage and synovium from patients with either OA or fracture to the neck of femur (NOF), giving a more complete picture of proteolysis in this disease.
Methods: Cartilage and synovium was collected at total hip replacement surgery and snap frozen in liquid nitrogen. Cartilage was ground under liquid nitrogen in a freezer mill and RNA purified by a combination of TRIzol (Invitrogen) and RNeasy (Qiagen). Synovium was homogenized into TRIzol using a Cell Lyser (Qiagen) and RNA purified. RNA was reverse transcribed and gene expression assessed using quantitative real-time PCR on the ABI Prism 7700.
Results: The four most significantly upregulated genes ($p<0.0001$) in OA synovium compared to NOF are *MMP28*, *ADAMTS16*, *ADAMTS17* and *TIMP2*. For *MMP9*, *MMP10*, *MMP12*, *MMP17, MMP23*, *MMP28*, *ADAMTS4, ADAMTS9* and *ADAMTS16*, there is a significant correlation between expression levels in the synovium and cartilage suggesting similar mechanisms of regulation. Additionally, we have shown that in cartilage, the median level of steady state mRNA for *MMP13* is approximately 20-fold higher than *MMP28* and approximately 1500-fold higher than *ADAMTS16* with this latter gene expressed approximately 150-fold higher in synovium than cartilage.
Conclusions: This is the most comprehensive analysis of gene expression of the metzincin family in the joint to date. It has identified several proteinase genes not previously reported to be expressed or regulated in the synovium.

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OMEGA-3 FATTY ACIDS REDUCE PRODUCTION OF PRO-INFLAMMATORY PROSTANOIDS, INCREASE PRODUCTION OF ANTI-INFLAMMATORY 15D-PGJ_2 AND ALTER LEVELS OF MMPS AND TIMPS BY OSTEOARTHRITIC SYNOVIAL FIBROBLASTS TREATED WITH BASIC CALCIUM PHOSPHATE CRYSTALS

B. McDonnell[1], E.S. Molloy[1], J. O'Byrne[2], M.P. Morgan[1], G.M. McCarthy[1]
[1]Royal College of Surgeons in Ireland, Dublin, Ireland, [2]National Orthopaedic Hospital, Cappagh, Dublin, Ireland

Purpose: The prevalence of basic calcium phosphate (BCP) crystals in synovial fluid from patients with knee osteoarthritis (OA) is between 30% and 60%. Intra-articular BCP crystals exert a pro-inflammatory effect through amplification of prostaglandin E_2 (PGE_2) and prostacyclin production in osteoarthritic synovial fibroblasts (OASF). They also upregulate the production of matrix metalloproteinases (MMPs) 1, 3, 9 and 13, which are involved in cartilage degradation. Transcription of tissue inhibitors of MMPs 1 & 2 (TIMP1 & 2) is downregulated after BCP treatment. Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are omega-3 fatty acids metabolised by cyclooxygenase enzymes. They appear to have anti-inflammatory effects in arthritis and other inflammatory diseases. We investigated the effect of omega-3 fatty acids on the production of prostaglandins, MMPs and TIMPs in OASF cells treated with BCP crystals.
Methods: OASF were obtained at the time of joint replacement surgery and cultured in monolayer. Cells were enriched with EPA or DHA (50μM) for 24hours prior to stimulation with BCP crystals (18$\mu g/cm^2$). Prostaglandin production was measured by enzyme immuno-assay. Changes in expression of MMPs and TIMPs were analysed using Real-Time PCR. All data was statistically analysed using the unpaired T-test.
Results: Treatment of BCP-stimulated OASF with EPA and DHA (50μM) resulted in a statistically significant ($p \leq 0.05$) reduction in PGE_2 and prostacyclin levels at 4hours. DHA treatment also resulted in a three-fold increase in production of 15d-PGJ_2, in contrast to a small increase (0.5 fold) in EPA treated cells. Increases in MMP production following BCP crystal stimulation is significantly attenuated by EPA and DHA treatment, with mRNA levels reduced to near those of cells unstimulated by BCP crystals. TIMP1 and 2 production following BCP stimulation is significantly increased following EPA treatment with levels increased to greater than those of unstimulated cells.
Conclusions: EPA and DHA reduce the pro-inflammatory effects of BCP crystals on OASF cells via a reduction in PGE_2. Production of prostacylin which is implicated in inflammation, nociception and angiogenesis is also reduced. The increased production of 15d-PGJ2 may enhance an anti-inflammatory effect through its role as an endogenous ligand for the intranuclear receptor PPARgamma and we propose to investigate this further. The increased levels of cartilage-degrading MMPs in BCP-stimulated cells are significantly reduced and the levels of TIMP1 and 2 are significantly higher following fatty acid treatment than in BCP-stimulated cells alone. These data highlight the potential beneficial effects of omega-3 fatty acids in BCP-crystal associated OA.